IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Mouritsen et al.
Serial No.	:	08/955,373
Filed	:	October 21, 1997
Examiner	:	Ron Schwadron
Art Unit	:	1644
For	:	**INDUCING ANTIBODY RESPONSE AGAINST SELF-PROTEINS WITH THE AID OF FOREIGN T-CELL EPITOPES**

745 Fifth Avenue, New York, New York 10151

SECOND DECLARATION OF PAUL J. TRAVERS, PhD

Assistant Commissioner for Patents
Washington, D.C. 20231
Dear Sir:

PAUL J. TRAVERS declares and says that:

1. I am the Paul Travers that executed a Declaration dated October 6, 2000 (my First Declaration) in the above-captioned application (the present application), which is hereby incorporated herein by reference, including the *Curriculum vitae* attached thereto, and advise that "Immunobiology - The Immune System In Health and Disease," by Charles Janeway, Jr. and Paul Travers. Garland Publishing, Inc. is now in its Fifth edition (2001) (also known as the "Janeway/Travers Immunology" textbook). Indeed, I am advised that some have called Immunobiology - The Immune System In Health and Disease," "the preeminent textbook Janeway/Travers Immunology" and characterized it as "probably the most widely known and recognized textbook in immunology in the world." Furthermore, in addition to having read and understood the disclosure in the present application, as discussed in my First Declaration, I am informed that a concurrently-filed Amendment presents claims as reproduced below or substantially as reproduced below, after my signature, which I have read and understood. Accordingly, in view of my education, training and experience, I respectfully submit that well qualified to speak as to the present application and the state of the art to which it pertains, contrary to any different assertions by the Examiner.

2. More specifically, I am informed that in the November 27, 2001 Office Action, the Examiner has asserted that I am not a person knowledgeable in the field of immunology or more specifically in the field relating to immunosuppression; and I have been informed that the

Examiner, in rejecting claims, has indicated that it would have been obvious for an immunologist to substitute suppressor epitopes in self-proteins with foreign T-helper epitopes or that an immunologist could have substituted suppressor epitopes in self proteins with T-helper epitopes. This Declaration is directly responsive to these points and the November 27, 2001 Office Action.

I AM QUITE QUALIFIED TO SPEAK AS TO THE PRESENT INVENTION

3. I respectfully submit that my credentials amply demonstrate that I am indeed "an expert in the particular technology relevant to the claimed invention," contrary to the Examiner's assertion in the November 27, 2001 Office Action. The Examiner, is, of course, respectfully invited to reconsider and withdraw his contrary assertions as to my credentials as set forth in the November 27, 2001 Office Action, particularly in view of matters discussed herein and other documents filed concurrently herewith. And the Examiner is also respectfully invited to make his credentials of record so that any reviewer of the file of the present application may compare and contrast his credentials with mine and those of any other declarants, so that the reviewer can judge for himself or herself who is best qualified to address the present invention and the field to which it pertains.

THE EXAMINER'S HYPOTHETICAL SUBSTITUTION OF SUPPRESSOR EPITOPES IS NOT AND WAS NOT POSSIBLE

4. I further respectfully submit that perhaps the Examiner and I have been miscommunicating; and, perhaps the Examiner's positions are based upon a failure of the nomenclature in the art.

5. More specifically, I understand that the Examiner has cited Example 5 of Russell-Jones, WO 92/05192, which involves HIV gp120 – a major surface antigen of HIV – and the hypothesis that one can replace "suppressor regions" therein. From this, I understand that the Examiner cites two Abstracts, Miller et al., J. Immunology 151(12):7307-7315 (1993) and Parkar et al., J. Immunol Methods 120(2):159-166 (1989), for the assertion that there are suppressor epitopes in mammalian proteins. I understand that the reliance on these Abstracts is to assert that "mammalian proteins containing suppressor regions/epitopes are well known in the art" and to attempt to discredit me as an expert.

6. I thus understand that the Examiner has equated the "suppressor regions" of gp120 with postulated, hypothetical "suppressor epitopes" in the Abstracts.

7. A full reading of Miller et al. shows that the same epitope can be either activating or suppressive depending on the route and nature of administration and that in general to speak of a 'suppressive epitope' in isolation of these other factors is inappropriate. However, my reading of the Examiner's comments is that he refers to epitopes which are intrinsically or constitutively suppressive. It is highly controversial whether such suppressor epitopes exist; but, more importantly, there is, to the best of my knowledge, no known method of positively identifying such suppressor epitopes. And at the August 26, 1993 effective filing date of the present application, to the best of my knowledge, there was no known method of positively identifying such suppressor epitopes. Indeed, note that Parkar, for example, is seeking to "facilitate the mapping of ... suppressor epitopes" indicating that there was no known method for positively identifying such suppressor epitopes. Note further Etlinger, "Carrier sequence selection – one key to successful vaccines," Immunology Today, Vol. 13 No. 2 pp 52-55 (copy attached) which also shows that there were no methods available for the identification of T-suppressor epitopes (*cf.* Etlinger at p 53, right-hand column, first full paragraph).

8. Simply, it is not possible today - and was not possible at the August 26, 1993 effective filing date of the present application - to devise any strategy for immunisation by substituting suppressor epitopes of self-proteins with foreign T-helper epitopes. Clearly, if one skilled in the art could not and cannot positively identify suppressor epitopes in self-proteins (whose existence is still a matter of debate in the art), based on my education, training and experience, there is no way and was no way for the skilled artisan to perform the hypothetical substitution postulated by the Examiner. Moreover, such substitutions would be ineffective in allowing immunisation against self-proteins unless the naturally expressed self-proteins were first removed, and this is beyond the current state of the art.

9. Moreover, if "suppressor epitopes" do exist, a suppressor epitope would activate suppressor T-Lymphocytes, whereas the "suppressor region" of gp120 is implicated in leading to a depletion of CD4+ cells (loss of T-helper cells and/or function). Hence, even if "suppressor epitopes" do exist, they are different than the gp120 suppressor region; and, their function would be totally different than that of the gp120 "suppressor region". Russell-Jones demonstrates that the suppressor peptide of gp120 provides for a general lack of immune responsiveness, i.e., an antigen unspecific suppression. In contrast, if such a thing as a specific T-suppressor epitope would exist, it would by nature provide for an antigen specific response, i.e., it would only

reduce the immune response against the very antigen comprising the epitope. Ergo, one could not equate or extrapolate from the gp120 "suppressor region" to the postulated suppressor epitopes, as I understand the Examiner had endeavored to do in the November 27, 2001 Office Action. If one follows the Examiner's reasoning that suppressor regions and suppressor epitopes are the "same thing", the art did and does not render it possible for the skilled person to identify a T-suppressor epitope and substitute this with a foreign immunodominant T-cell epitope (and also preserve tertiary or secondary and tertiary structure, since the position of such a suppressor epitope may be essential for the structure of the protein). Hence, one cannot follow the Examiner's reasoning and arrive at the present invention. Furthermore, even though Russell-Jones at page 32 postulates that "[u]sing recombinant DNA technology, the 'suppressor regions' in a number of prospective vaccine proteins including gp120 are removed and replaced," this is not a teaching or suggestion that would or could lead to the instant invention. Simply, as demonstrated herein, the gp120 suppressor region is not the same as or equivalent to the postulated suppressor epitopes, and one cannot extrapolate from the gp120 suppressor region to the postulated suppressor epitopes. Moreover, Russell-Jones at page 32 addresses "vaccine proteins". Self-proteins are not "vaccine proteins". "Vaccine proteins" are normally immunogenic. "Vaccine proteins" are not normally non-immunogenic. There is not normally B-cell autotolerance to "vaccine proteins". Hence, the statement at page 32 of Russell-Jones does not permit extrapolation to self-proteins. In addition, I respectfully submit that the Examiner's positions may be borne out of a failure in the nomenclature in the art and/or a miscommunication between him and me. In any event, I trust that this Declaration clarifies the nomenclature in the art and any possible miscommunication that may have occurred. It is hoped that with this Declaration the Examiner appreciates that Russell-Jones, either individually or in any combination fails to teach or suggest the instant invention.

THE EXAMINER'S APPLICATION OF RUSSELL-JONES STRAINS THE TERM "IMMUNOGEN" WELL BEYOND ITS WELL-KNOWN, ORDINARY, ART-ACCEPTED DEFINITION

10. I additionally understand that in the November 27, 2001 Office Action, the Examiner relies upon the text at pages 8-9 of Russell-Jones for applying that document as to the present invention. More specifically, the text at pages 8-9 of Russell-Jones states: "The at least one 'immunogen' which forms part of a complex of the invention is any molecule which it is

desirable to use to raise an immune response. Typically, the at least one 'immunogen' will be a molecule which is poorly immunogenic, but immunogenic molecules are not excluded." From this, the Examiner, I understand, asserts that a self-protein – a protein that is normally non-immunogenic and as to which there is B-cell autotolerance – can be an "immunogen".

11. By definition an "immunogen" – whether "immunogenic" or "poorly immunogenic" – elicits an immune response, and there is no B-cell autotolerance thereto; i.e., by definition, a self-protein is not an immunogen. If a self-protein is an immunogen, there would not be B-cell autotolerance thereto, and, in essence, the body would be fighting itself – mounting an immunological response against self-proteins. The plain, ordinary, and art-recognized definition of "immunogen" and the plain, ordinary, and art-recognized reading of Russell-Jones, excludes self-proteins, and is contrary to the Examiner's reading of Russell-Jones and his expansion of the term "immunogen". Hence, the Examiner's reading of Russell-Jones and his expansion therein of the term "immunogen" strain the term "immunogen" beyond its ordinary, art-recognized, well-known meaning.

12. Furthermore, the mention in Russell-Jones of luteinizing hormone, somatostatin, inhibin and FSH do not allow expansion of the term "immunogen" to include self-proteins, as I understand is attempted by the Examiner. I also understand that the Examiner asserts that: "[t]here is no teaching in Russell-Jones et al. that humans would be immunized with nonhuman modified luteinizing hormone, somatostatin, inhibin or FSH. ... The use of human derived molecules in vaccines for humans was known in the art. ... [V]irtually any self molecule is an immunogen if administered to another species of animal or if administered to the animal from which it was derived wherein it is administered with an appropriate adjuvant. ... Russell-Jones et al. teach that it would be within the skill of a routineer to produce modified fusion proteins wherein Trat was included and wherein the fusion protein still had the activity of the parent molecule."

13. One could not use an unmodified human self-protein in a human to elicit an immunological response against the self-protein because the self-protien is non-immunogenic; there is normally B-cell autotolerance to the self-protein. Thus, it is common to use non-human analogs of human self-proteins in humans, to avoid the non-immunogenicity of and B-cell autotolerance to the self-protein. Hence, from the disclosure in Russell-Jones of luteinizing hormone, somatostatin, inhibin and FSH, one cannot extrapolate that human self-proteins are

intended for humans because the same disclosure more than equally indicates to the skilled artisan the use of non-human analogs of human self-proteins in humans. Indeed, non-human analogs of human self-proteins in humans are consistent with Russell-Jones' use of the term "poorly immunogenic" because non-human analogs of human self-proteins in humans are known to be poorly immunogenic, rather than non-immunogenic (a feature of self-proteins). Thus, the skilled artisan, from fully reading Russell-Jones, would not be led to modifying self-proteins. Also, the skilled artisan does not view the term "immunogen" as encompassing a self-protein, which is non-immunogenic and to which there B-cell autotolerance, "administered with an appropriate adjuvant." This too strains the art-recognized, well-known, and accepted definition of "immunogen."

14. It is respectfully submitted that it is unclear as to what is meant by the statement "Russell-Jones et al. teach that it would be within the skill of a routineer to produce modified fusion proteins wherein Trat was included and wherein the fusion protein still had the activity of the parent molecule." And it is unclear how this statement pertains to the present invention. More specifically, Russell-Jones involves modifying immunogens or antigens – proteins which are normally immunogenic and are not normally non-immunogenic and are not normally B-cell autotolerated – to produce fusion proteins thereof. The "activity of the parent molecule" is eliciting an immune response. In contrast, the present invention involves modified self-proteins which are normally non-immunogenic and to which there is normally B-cell autotolerance, i.e., "the activity" of the self-protein is not eliciting an immune response. The modification of the self-protein in the instant invention is so that in comparison to the self-protein, the modified self protein contains at least one foreign T-cell epitope; the modified self-protein of the instant invention is not a fusion protein. Other parameters of the instant invention are set forth in the claims that appear below my signature. Hence, the modified self-protein does not have the activity of the self-protein, and is not a fusion protein. Accordingly, the foregoing statement from the November 27, 2001 Office Action, in my view, is unclear, especially as to how it may pertain to the instant invention.

15. Clearly, therefore, Russell-Jones, either individually or in any combination, fails to teach or suggest the instant invention. Indeed, it is particularly noted that the claims that appear below my signature call for methods for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-

protein of that animal; and, from my reading of Russell-Jones, based upon my education, training and experience in the field to which the present invention pertains, I do not see any teaching or suggestion of such methods, or any teachings, suggestions, motivations, or incentives to modify Russell-Jones to arrive at methods for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal.

16. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 16 MAY 2002 By: _____
PAUL J. TRAVERS, PhD

CLAIMS UNDERSTOOD TO BE ADDED OR SUBSTANTIALLY ADDED

--56. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein;

whereby, the modified self-protein elicits antibodies that are against the self-protein, and B-cell autotolerance to the self-protein is broken.

57. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein;

whereby the modified self-protein elicits antibodies that are against the self-protein, and B-cell autotolerance to the self-protein is broken.

58. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least four amino acids on each side of the peptide fragment;

whereby the modified self-protein elicits antibodies that are against the self-protein, and B-cell autotolerance to the self-protein is broken.

59. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving

tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least ten amino acids on each side of the peptide fragment;

whereby the modified self-protein elicits antibodies that are against the self-protein, and B-cell autotolerance to the self-protein is broken.

60. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least fifteen amino acids on each side of the peptide fragment,

whereby the modified self-protein elicits antibodies that are against the self-protein, and B-cell autotolerance to the self-protein is broken.

61. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein,

whereby the modified self-protein elicits antibodies that are against the self-protein; and, the modified self-protein elicits an immune response in the animal which includes an MHC class II immune response as to the immunodominant T-cell epitope and an autoantibody response in other MHC-haplotypes, and B-cell autotolerance to the self-protein is broken.

62. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by being detoxified and by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein,

whereby the modified self-protein elicits antibodies that are against the self-protein, and B-cell autotolerance to the self-protein is broken.

63. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least four amino acids on each side of the peptide fragment;

whereby the modified self-protein elicits antibodies that are against the self-protein, and B-cell autotolerance to the self-protein is broken.

64. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least ten amino acids on each side of the peptide fragment;

whereby the modified self-protein elicits antibodies that are against the self-protein, and B-cell autotolerance to the self-protein is broken.

65. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least fifteen amino acids on each side of the peptide fragment,

whereby the modified self-protein elicits antibodies that are against the self-protein, and B-cell autotolerance to the self-protein is broken.

66. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein,

whereby the modified self-protein elicits antibodies that are against the self-protein; and, the modified self-protein elicits an immune response in the animal which includes an MHC class II immune response as to the immunodominant T-cell epitope and an autoantibody response in other MHC-haplotypes, and B-cell autotolerance to the self-protein is broken.

67. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by being detoxified and by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein,

whereby the modified self-protein elicits antibodies that are against the self-protein, and B-cell autotolerance to the self-protein is broken.

68. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, comprising;

preparing different modified self-proteins, wherein:

each modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal,

said substitution preserving tertiary structure of the self-protein, and

the different modified self-proteins differ from each other with respect to the position of the at least one immunodominant T-cell epitope;

ascertaining which of the different modified self-proteins elicits a desired specific neutralizing effect and thereby ascertaining a desired modified self protein; and

administering to the animal, an immunologically effective amount of the desired modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein, and,

the desired modified self-protein elicits antibodies that are against the self-protein, and B-cell autotolerance to the self-protein is broken.

69. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, comprising;

preparing different modified self-proteins, wherein:

each modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal,

said substitution preserving secondary and tertiary structure of the self-protein, and

the different modified self-proteins differ from each other with respect to the position of the at least one immunodominant T-cell epitope;

ascertaining which of the different modified self-proteins elicits a desired specific neutralizing effect and thereby ascertaining a desired modified self protein; and

administering to the animal, an immunologically effective amount of the desired modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein, and,

the desired modified self-protein elicits antibodies that are against the self-protein, and B-cell autotolerance to the self-protein is broken.

70. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

a. the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein;

whereby, the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

b. the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein;

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

c. the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least four amino acids on each side of the peptide fragment;

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

d. the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least ten amino acids on each side of the peptide fragment;

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

e. the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least fifteen amino acids on each side of the peptide fragment,

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

f. the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein,

whereby the modified self-protein elicits antibodies that are against the self-protein earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes; and, the modified self-protein elicits an immune response in the animal which includes an MHC class II immune response as to the

immunodominant T-cell epitope and an autoantibody response in other MHC-haplotypes, and B-cell autotolerance to the self-protein is broken; or,

g. the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by being detoxified and by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein,

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

h. the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least four amino acids on each side of the peptide fragment;

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

i. the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein, and said substitution

preserving flanking regions comprising at least ten amino acids on each side of the peptide fragment;

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

j. the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least fifteen amino acids on each side of the peptide fragment,

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

k. the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein,

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes; and, the modified self-protein elicits an immune response in the animal which includes an MHC class II immune response as to the immunodominant T-cell epitope and an autoantibody response in other MHC-haplotypes, and B-cell autotolerance to the self-protein is broken; or,

1. the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by being detoxified and by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein,

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

m. preparing different modified self-proteins, wherein:

each modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal,

said substitution preserving tertiary structure of the self-protein, and

the different modified self-proteins differ from each other with respect to the position of the at least one immunodominant T-cell epitope;

ascertaining which of the different modified self-proteins elicits a desired specific neutralizing effect and thereby ascertaining a desired modified self protein; and

administering to the animal, an immunologically effective amount of the desired modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein, and,

the desired modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

n. preparing different modified self-proteins, wherein:

each modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal,

said substitution preserving secondary and tertiary structure of the self-protein, and

the different modified self-proteins differ from each other with respect to the position of the at least one immunodominant T-cell epitope;

ascertaining which of the different modified self-proteins elicits a desired specific neutralizing effect and thereby ascertaining a desired modified self protein; and

administering to the animal, an immunologically effective amount of the desired modified self-protein, wherein:

the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein, and,

the desired modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken.

71. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, inducing antibody production in the animal against the self-protein of that animal, and eliciting an immune response in the animal which includes an MHC class II immune response as to an immunodominant T-cell epitope which is foreign to the animal and an autoantibody response in other MHC-haplotypes, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

a. the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing the immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein,

whereby the modified self-protein elicits antibodies that are against the self-protein; and, the modified self-protein elicits an immune response in the animal which includes an MHC class II immune response as to the immunodominant T-cell epitope and an autoantibody response in other MHC-haplotypes, and B-cell autotolerance to the self-protein is broken; or

b. the self-protein is normally autotolerated by the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing the immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein,

whereby the modified self-protein elicits antibodies that are against the self-protein; and, the modified self-protein elicits an immune response in the animal which includes an MHC class II immune response as to the immunodominant T-cell epitope and an autoantibody response in other MHC-haplotypes, and B-cell autotolerance to the self-protein is broken.

72. (New) A method for breaking B-cell autotolerance in an animal to a self-protein of that animal, and inducing antibody production in the animal against the self-protein of that animal, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, comprising administering to the animal, an immunologically effective amount of at least one modified self-protein, wherein:

a. the self-protein is normally non-immunogenic in the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein;

whereby, the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

b. the self-protein is normally non-immunogenic in the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein;

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

c. the self-protein is normally non-immunogenic in the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least four amino acids on each side of the peptide fragment;

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

d. the self-protein is normally non-immunogenic in the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least ten amino acids on each side of the peptide fragment;

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

e. the self-protein is normally non-immunogenic in the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said

substitution preserving tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least fifteen amino acids on each side of the peptide fragment,

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

f. the self-protein is normally non-immunogenic in the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein,

whereby the modified self-protein elicits antibodies that are against the self-protein earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes; and, the modified self-protein elicits an immune response in the animal which includes an MHC class II immune response as to the immunodominant T-cell epitope and an autoantibody response in other MHC-haplotypes, and B-cell autotolerance to the self-protein is broken; or,

g. the self-protein is normally non-immunogenic in the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by being detoxified and by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving tertiary structure of the self-protein,

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

h. the self-protein is normally non-immunogenic in the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least four amino acids on each side of the peptide fragment;

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

i. the self-protein is normally non-immunogenic in the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least ten amino acids on each side of the peptide fragment;

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

j. the self-protein is normally non-immunogenic in the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein, and said substitution preserving flanking regions comprising at least fifteen amino acids on each side of the peptide fragment,

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

k. the self-protein is normally non-immunogenic in the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein,

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes; and, the modified self-protein elicits an immune response in the animal which includes an MHC class II immune response as to the immunodominant T-cell epitope and an autoantibody response in other MHC-haplotypes, and B-cell autotolerance to the self-protein is broken; or,

1. the self-protein is normally non-immunogenic in the animal and there is normally B-cell autotolerance by the animal to the self-protein; and,

the modified self-protein is modified, in comparison to the self- protein, by being detoxified and by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal, said substitution preserving secondary and tertiary structure of the self-protein,

whereby the modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

m. preparing different modified self-proteins, wherein:

each modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal,

said substitution preserving tertiary structure of the self-protein, and

the different modified self-proteins differ from each other with respect to the position of the at least one immunodominant T-cell epitope;

ascertaining which of the different modified self-proteins elicits a desired specific neutralizing effect and thereby ascertaining a desired modified self protein; and

administering to the animal, an immunologically effective amount of the desired modified self-protein, wherein:

the self-protein is normally non-immunogenic in the animal and there is normally B-cell autotolerance by the animal to the self-protein, and,

the desired modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken; or,

n. preparing different modified self-proteins, wherein:

each modified self-protein is modified, in comparison to the self- protein, by containing a substitution of at least one peptide fragment of the self-protein with a peptide containing at least one immunodominant T-cell epitope which is foreign to the animal,

said substitution preserving secondary and tertiary structure of the self-protein, and

the different modified self-proteins differ from each other with respect to the position of the at least one immunodominant T-cell epitope;

ascertaining which of the different modified self-proteins elicits a desired specific neutralizing effect and thereby ascertaining a desired modified self protein; and

administering to the animal, an immunologically effective amount of the desired modified self-protein, wherein:

the self-protein is normally non-immunogenic in the animal and there is normally B-cell autotolerance by the animal to the self-protein, and,

the desired modified self-protein elicits antibodies that are against the self-protein, earlier and in higher titres, in comparison to the self-protein conjugated to a carrier protein or peptide containing T-cell epitopes, and B-cell autotolerance to the self-protein is broken.

73. (New) The method of any one of claims 56-72 wherein the modified self-protein is a recombinant modified self-protein.

74. (New) The method of any one of claims 56-72 wherein the self-protein is tumor necrosis factor alpha (TNF-α), tumor nucrosis factor beta (TNF-β), gamma interferon (γ-interferon), interleukin 1 (IL-1) or immune globulin (IgE).

75. (New) The method of claim 73 wherein the self-protein is tumor necrosis factor alpha (TNF-α), tumor nucrosis factor beta (TNF-β), gamma interferon (γ-interferon), interleukin 1 (IL-1) or immune globulin (IgE).

76. (New) The method of any one of claims 56-72 wherein the administering includes administering an adjuvant.

77. (New) The method of claim 76 wherein the adjuvant comprises calcium phosphate, saponin, quil A or a biodegradable polymer.

78. (New) The method of claim 73 wherein the administering includes an adjuvant.

79. (New) The method of claim 75 wherein the administering includes an adjuvant.--

Carrier sequence selection – one key to successful vaccines

Howard M. Etlinger

The trend towards epitopic vaccines has brought with it the problem of ensuring carrier function, a role previously filled by carrier sequences of the attenuated organism. Here, Howard Etlinger proposes the use of carrier epitopes, derived from vaccines already in use and selected for their ability to activate only helper T-cell responses, in the administration of B-cell-specific epitopic vaccines.

It has been three millennia since material from smallpox pustules was first used successfully as a vaccine[1]. In the last 300 years, living, wild-type vaccines have been replaced by attenuated or killed forms of pathogens. The recent identification of protective portions of pathogens permits the development of new vaccines that use only subunits of the pathogen or sequences representing protective epitopes in the form of recombinant proteins, recombinant microorganisms, synthetic peptides or internal image antibody[2–4].

The advantages of vaccines that do not use whole organisms include a reduced risk of contaminants and virulence, and increased availability of vaccine components. In addition, since a polypeptide might contain both protective and suppressive epitopes[5], the ability to specify the primary structure of a vaccine by recombinant DNA techniques or peptide synthesis provides the opportunity of excluding such inhibitory sequences. The disadvantages of epitopic vaccines include the potential difficulties in obtaining the structure essential for protective immunity (ways around this potential problem have been discussed[4,6–8]) and the need to identify protective epitopes and effective adjuvants.

In this article a new approach to the problems raised by epitopic vaccines, which takes advantage of the widespread use of vaccines, is presented. This approach depends on the recognition of a helper T-cell (T_H-cell) peptide sequence from a vaccine protein by most of the population. Since the effect of preimmunization with carrier protein in humans is variable and can be inhibitory, this approach is designed to take advantage of primed T_H cells, while at the same time excluding potentially inhibitory lymphocytes arising from prior vaccinations. It envisions linking poorly immunogenic, protective B-cell sequences to a carrier sequence from a common, current vaccine. The best way to prevent the involvement of inhibitory B or T cells is to use a carrier peptide that is recognized by helper T cells but not by B cells or suppressor T cells in vaccinated (carrier-primed) individuals.

Carrier sequence selection

The original method used to obtain peptides was to chemically or enzymatically hydrolyse a protein and purify the resulting peptides for testing. Subsequently, algorithms were developed that predict the portions of a protein likely to contain T-cell epitopes[9,10]. This approach is very useful but is essentially based on peptides that elicit proliferative responses and some carrier sequences can provide good helper function without inducing proliferation[11–13]. Recent analyses of self peptides isolated from major histocompatibility complex (MHC) class I molecules have revealed allele-specific motifs[14]. Although peptides presented by MHC class II molecules[10,15] are not so rigorously selected[16], it may be important to consider sequence motifs to ensure general recognition of the carrier peptide (see below). If no assumptions are made about which sequences are likely to work, and the only requirement is that the amino acids are continuous, then peptides covering the entire length of the protein can be synthesized for evaluation. This empirical approach can identify stimulatory peptides that are not described by algorithms[15].

Peptides can be screened for potential helper activity using T-cell proliferation assays with lymphocytes from mice primed with the vaccine protein or with peripheral blood lymphocytes from vaccinated humans. The MHC class II diversity of the mice and humans used in this screening phase helps to ensure that the peptide will be generally recognized. To determine helper function directly, a sequence containing only B-cell epitopes can be linked to the test sequence and the resultant immunogen evaluated for its ability to elicit an antibody response to the B-cell sequence. For example, BALB/c mice are genetically low responders to $(Asn\text{-}Ala\text{-}Asn\text{-}Pro)_n$ $(NANP)_n$ at the T-cell level[17,18] but produce good antibody responses to $(NANP)_n$ when this peptide is linked to a peptide containing amino acid residues 73–99 of tetanus toxoid (TT73–99) ($(NANP)_4$TT73–99) (Ref. 19).

Epitope-specific suppression

A sequence representing a protective B-cell epitope may not contain information for effective carrier function. In this case, carrier epitopes need to be included. So far, carriers for human vaccines have been proteins that are themselves used as vaccines – tetanus toxin and diphtheria toxin. Although some success has been achieved[20,21], there is evidence that prior immunity to these carrier proteins inhibits antibody responses to the new B-cell epitopes[22,23]. The basis for inhibition may be epitope-specific suppression. This occurs when an animal is immunized with a protein, and is then challenged with the same protein (now functioning as a carrier) to which hapten has been coupled. The distinctive feature is selective inhibition of the IgG anti-hapten response, even upon subsequent immunization with the same hapten on an unrelated carrier[24–26].

Epitope-specific suppression was originally described *in vivo*[26,27] but, since it is difficult to reproduce by adoptively transferring cells into irradiated recipients[27], the phenomenon has largely been studied *in vitro*[28–30]. *In vivo* and *in vitro* findings are not identical: in the animal, antibody responses to the carrier are not inhibited by carrier priming (hence are 'epitope specific'), whereas, *in vitro*, they are, although less markedly than the anti-hapten responses[28,29].

The mechanism of inhibition is multifaceted. Carrier-primed spleen cells transferred into nonirradiated recipients selectively suppress the anti-hapten response; depletion of T cells from the transferred cell population abrogates suppression[26]. This suggests that carrier-specific suppressor T cells are involved and it was proposed that such cells induce hapten-specific suppressor T cells that either directly inhibit B-cell responses or induce cells that mediate suppression. *In vitro*, Tagawa *et al.*[28] showed that anti-hapten responses are inhibited by both Lyt-2$^+$ and Lyt-2$^-$ T cells; a second study found that both Lyt-2$^+$ and L3T4$^+$ T cells, as well as B cells, are suppressive[29]; whereas a third *in vitro* study[30] identified an additional mechanism for epitope-specific regulation: inhibition of the anti-hapten response was localized to an intrinsic defect (clonal anergy) in hapten-specific B cells, so that they could not secrete IgG following challenge with a T-cell-dependent antigen. There was no evidence for B-cell-mediated suppression and T-cell-mediated suppression was not assessed[30].

It is possible that unprimed, hapten-specific B cells that interact with antigen without a sufficient level of help (caused by suppressive T cells, see below) become anergic. An alternative, or additional, explanation is that other B cells mediate epitope-specific regulation. Exactly how this might occur is not clear, because, to date, it has been evaluated using hapten coupled to the same carrier as that used to elicit suppression[29]. It is possible that carrier-primed B cells inhibit by competing with unprimed hapten-specific B cells for antigen, but this is not consistent with published data[31,32]. Regardless of the mechanism by which B cells mediate suppression, a carrier protein that is not recognized by carrier-primed B cells will not be susceptible to such regulation. However, there may be practical difficulties in determining whether a candidate carrier peptide is recognized by primed B cells; the best option is to test sera for anti-carrier antibody. In the example cited above, human or mouse sera obtained after vaccination with TT or $(NANP)_3$TT were tested for reactivity with TT73–99; neither sera reacted with this peptide[19].

Both Lyt-2$^+$ and Lyt-2$^-$ T cells are involved in suppression[24,28]. The carrier-specific helper T cell that induces hapten-specific suppressor T cells[26] could be a CD4$^+$ 'suppressor–inducer' T cell (T_{SI}[33,34]) and, from the standpoint of carrier sequence selection, it is important to know whether CD4$^+$ T_{SI} cells recognize the same peptides as helper T cells do; this might be the case, since there is evidence that helper T cells give rise to T_{SI} cells[5]. In addition, CD4$^-$ T cells could also function as effector cells, since subpopulations of CD4$^+$ helper T cells, T_H1 and T_H2 cells, can mutually inhibit each other[35]. T_H1 cells provide help for IgG2a but not IgG1 responses, whereas the converse is true for T_H2 cells. Under conditions of epitope-specific suppression, IgG2a responses are typically inhibited more than IgG1 responses are[27], which is consistent with a role for T_H2 cells; a caveat for this mechanism, at least *in vivo*, is the hapten specificity of suppression. As is the case for T_{SI} cells, should some of the Lyt-2$^-$ (Ref. 28 or Ly3T4$^+$ (Ref. 29) T cells that mediate suppression turn out to be T_H2 cells, it is important to know whether their specificity differs from that of T_H1 cells.

The mechanisms by which CD4$^+$ T_{SI} cells and CD8$^+$ suppressor T cells might function and the nature of suppressor T-cell epitopes have been reviewed[5]. It is not yet possible, on the basis of sequence alone, to distinguish helper from CD8$^-$ suppressor T-cell sequences, and it remains to be clarified whether a particular sequence elicits only help or suppression. The portions of a protein recognized by helper and suppressor (CD8$^+$) T cells are usually different, although, as in the case of L-tyrosine-*p*-azobenzenearsonate[36], they may be very similar. Perhaps the full definition of consensus motifs, even with the superimposed complexity arising from allele specificity, will permit accurate forecasting of helper and suppressor peptides.

At present, only indirect tests are useful for assaying suppressor T-cell recognition of a peptide. Such tests determine the effect of carrier-priming on the antibody response to a B-cell epitope linked to either the candidate helper peptide or the entire carrier protein. If the former is not recognized by carrier-specific suppressor T cells (or T_{SI} or carrier-primed B cells), but is recognized by carrier-primed helper T cells, then an enhanced rather than a reduced antibody response is anticipated. Such a response profile has been observed: in BALB/c mice preimmunized with TT and challenged with $(NANP)_3$TT or $(NANP)_4$TT73–99, the anti-$(NANP)_n$ response to $(NANP)_3$TT is inhibited and the response to $(NANP)_4$TT73–99 is enhanced[19]. Importantly, the primary response in TT-primed mice is enhanced; this could be useful in achieving a quick protective response. To demonstrate the potential for general circumvention of epitope suppression in humans, this analysis should be performed in mice with different MHC haplotypes. Which suppressive cell type is active in this TT model[19] is not known, but it is possible to distinguish functionally sequences recognized by helper and suppressor cells. Even if helper and suppressor specificities turn out to be the same, the method of immunization, including vaccine formulation (for example choice of adjuvant), might be exploited to prime selectively the desired lymphocyte subpopulations[15].

Using the carrier peptide recursively

For this approach to be practical, the carrier peptide sequence must be 'reusable'. That is, after being vaccinated with a carrier protein and then with a B-cell epitope linked to a carrier peptide, an individual should still produce enhanced responses to further B-cell epitopes linked to the same carrier peptide. This has worked in a model system in which BALB/c mice were immunized with TT, then with a carrier peptide (TT88–99) linked to

$(NANP)_4$ and then with a peptide consisting of amino acid residues 46–61 from hen egg-white lysozyme (HEL46–61) linked to TT88–99 (Ref. 37). Good antibody responses were obtained to HEL46–61, which, like $(NANP)_n$, is poorly seen by BALB/c helper T cells. It appears that, as new protective B-cell epitopes are identified, it will be possible to link them to a previously used carrier sequence to achieve immunity in a given individual against new pathogens.

Protection and priming

An ideal vaccine elicits protective immunity and memory so that subsequent exposure to the pathogen will result in elimination of the pathogen and in boosted immunity. The approach discussed here is primarily intended to enhance the immunogenicity of poorly immunogenic, protective B-cell determinants.

Encouragingly high levels of protection have been achieved in a rodent malaria model against the sporozoite stage of the parasite, *Plasmodium berghei*[38]. Mice vaccinated with a TT conjugate containing a 17-mer peptide sequence from the tandem repeat region of the major surface protein were protected to the same degree (about 80% of the mice had sterile immunity) as animals immunized with irradiated sporozoites.

The importance of priming or memory induction by a vaccine has been discussed[3]. Since immunologic memory resides in both T and B cells[39–41] and a vaccine based on the present approach would not prime parasite-specific helper T cells, the central question is whether primed B cells alone can lead to a secondary response after exposure to a pathogen. Some results from animal studies suggest that B-cell priming alone is sufficient to obtain a secondary response, while others indicate that T-cell priming is essential[25,26,42,43]. Most interestingly, in a clinical study, a child received diphtheria toxoid coupled with oligosaccharides from the capsular polysaccharide of *Haemophilus influenzae* b, which presumably primes pathogen-specific B, but not helper T, cells. Three months after vaccination, the antibody titer to the oligosaccharide of the subject had declined from the peak level seen at one month but then increased more than 100-fold without further immunization, presumably as a result of colonization with bacteria. A significant portion of this antibody was composed of clonotypes that had previously been activated by the conjugate[44]. Thus, it is possible that a vaccine that sensitizes pathogen-specific B, but not helper T, cells can prime for an anti-parasite response. However, this may not be a general feature and will require evaluation on an individual basis.

General recognition of the helper sequence

Some vaccines are used worldwide and others are approaching this distribution. These vaccines, which serve as the grist for deriving helper T-cell sequences, include diphtheria toxoid, pertussis, TT, Bacile Calmette-Guérin (BCG), polio, measles, mumps and rubella. Generally recognized helper sequences from vaccines that could be used to help eliminate the problem of genetic restriction have been described for TT[45,46] and PPD (purified protein derivative of tuberculin)[47]. To ensure generic responsiveness it may be necessary to use more than one helper sequence, although it will need to be shown that the epitopes in such sequences are functional (minimal epitope dominance[48,49]) and that new suppressive determinants are not produced. If intramolecular antigenic competition arising from multiple helper sequences on a single peptide turns out to be a serious problem, then separate molecules can be used. However, even this strategy may not preclude intermolecular antigenic competition, which will need to be evaluated. Additional factors, such as the polarity of the helper T- and B-cell sequences[50] and the choice of adjuvant[51,52], will be important for the success of this approach. In the end, the intent is to improve upon the type of response which nature exhibits to tetanus which, as an infectious disease, "... is unique ... in that it is not communicable from person to person, and there is no natural immunity upon recovery"[53].

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